

Pycnidial Release and Survival of *Diplodia natalensis* Spores

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ABSTRACT

Hyaline, single-celled spores of *Diplodia natalensis* were released from pycnidia growing on citrus deadwood when the pycnidia became wet or when they dried following wetting. Discharge occurred during or after rainfall for 1 or 2 summers, depending upon the development of the pycnidia at the completion of the first season. After discharge from the pycnidium, single-celled spores differentiated into darker-colored two-celled spores within 5 to 6 hr at 100% relative humidity. This process was interrupted when

spores were placed at humidities below 100%, but continued upon return to 100% relative humidity. The two-celled spores were more tolerant to desiccation than the single-celled spores, and survived from 1 season to the next on the bark of naturally infected deadwood. As a result of the survival of these two-celled spores, infection of immature citrus fruit could occur even though pycnidia were not releasing spores. *Phytopathology* 61:559-561.

Additional key words: postharvest stem-end rot of citrus fruit.

Diplodia natalensis Pole-Evans causes a postharvest decay (stem-end rot) of Florida citrus that may result in devastating losses. This decay is particularly prevalent in fruit which have been degreened with ethylene to improve external color, and which are harvested from early-maturing cultivars (7). Though decay is not manifest until after harvest, infection occurs in the grove. Spores from pycnidia produced in deadwood of the tree are disseminated in water to necrotic tissue of the button (calyx and disc) of the immature fruit (3, 5, 8, 13). Hyphae from these latent infections invade the mature fruit through the abscission zone when the button abscises, which usually occurs after harvest (4, 5).

Spores of *D. natalensis* at pycnidial discharge are predominately single-celled and hyaline (immature), but occasionally are two-celled and dark brown (mature) (9, 10, 11, 14). Both types will germinate (9) and cause infection (2). Single-celled spores germinate in 2 to 6 hr (9, 11) in water or moist air (1), and are sensitive to drying after discharge (2). If not killed, the spores soon become two-celled (1) and remain viable for several days under laboratory conditions (2).

Further details on climatic factors that affect release and survival of the two different spore types were obtained in this study to elucidate the process of infection of immature citrus fruit by *D. natalensis*.

MATERIALS AND METHODS.—Spores for germination studies were obtained by growing *D. natalensis* on sterilized, nonliving twigs of Valencia orange, *Citrus sinensis* (L.) Osbeck and Duncan grapefruit, *C. paradisi* Macfadyen (4). A uniform discharge of single-celled spores was obtained from pycnidia when these twigs were covered with water for 5 min and dried. These spores developed into the two-celled form when the twigs were placed in moist chambers after drying. Tendrils of spores, observed with a stereoscopic microscope, were removed from the pycnidia with a scalpel. Pure spore suspensions were obtained when these spores were washed, concentrated by centrifugation (5 min at 2,340 g), and adjusted to $2-3 \times 10^5$ /ml in 1,000 ppm Tween 20 (polyoxyethylene sorbitan monolaurate).

Spores were removed from naturally infected dead twigs by agitating the twigs for 1 hr in 1,000 ppm Tween 20. The suspension was filtered through cheesecloth to remove debris, then washed and concentrated by centrifugation (5 min at 2,340 g).

Spores were germinated in a medium prepared from clipped Hamlin or Pineapple oranges. The cut surface of the stems 0.3-0.6 cm in length was sealed with paraffin, and the fruit were inverted in a shallow pan. Distilled water was added until the stem and button of the fruit were submerged. The water was removed 14 hr later, filtered through filter paper, autoclaved, and refrigerated. Spores were suspended in this medium with 1,000 ppm Tween 20. A drop of the spore suspension was placed on 22-mm square glass cover slips which were dried at 25 C and incubated in desiccators at high relative humidities controlled with salt solutions (12). Spore survival studies at lower relative humidities which did not sustain germination were handled similarly. Spores removed from the desiccators were covered with a drop of the medium and incubated at 30 C for 6 hr. Germination was terminated by adding a drop of aniline blue in lactophenol to the spore suspension. Relative humidities were verified with a Honeywell humidity and temperature meter.

RESULTS.—*Spore release.*—Pycnidia in fully developed stromata on dead twigs collected during February and March of 1970 contained few spores. These pycnidia could not be induced to produce and discharge spores during 6 weeks of alternate wetting or drying or continuous incubation in a moist chamber at 25 C. Newly formed pycnidia, present on deadwood collected the following June and July, were immersed in the host tissues and were difficult to detect; but they were actively discharging spores. Two-celled spores were present on the bark surface (Fig. 1, above) and in tendrils surrounding the ostiole of the pycnidium. Single-celled spores were discharged within 5 min when pycnidia on deadwood collected 3 days after rainfall were submerged in water. Pycnidia on wet, dead twigs collected in the morning after 3 successive days of rain immediately discharged numerous tendrils of single-celled

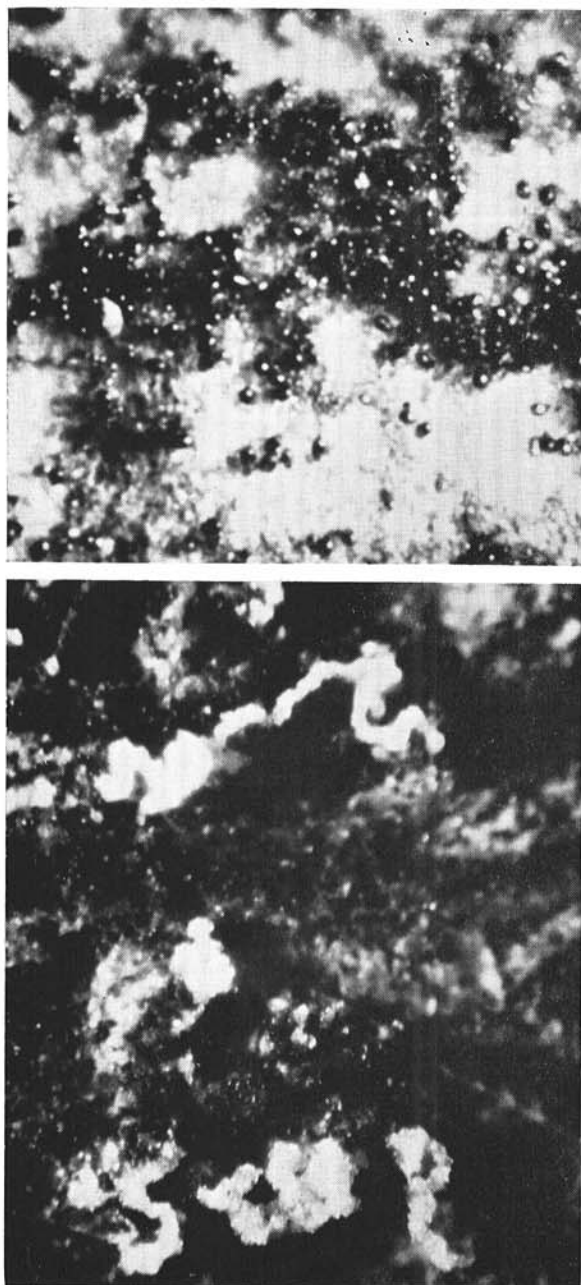


Fig. 1. (Above) Dark-colored two-celled spores of *Diplodia natalensis* on the bark surface of citrus deadwood adjacent to pycnidia ($\times 150$ and $\times 3$). (Below) Tendrils of hyaline single-celled spores released from pycnidia during drying ($\times 44$ and $\times 5$).

spores during drying (Fig. 1, below). Pycnidia in not yet fully developed stromata, which were produced the latter part of the previous summer, also released single-celled spores under similar conditions.

Spore survival.—Single-celled spores exuded in tendrils rapidly became two-celled on twigs or glass cover slips when they were maintained at 100% relative humidity at 25 C. Within 5 to 6 hr, the hyaline, nonsep-

tate, and granular spores (Fig. 2-A) became light gray, developed septa, and exhibited large oil vacuoles in each of the two cells (Fig. 2-B). After 24 hr, these spores were dark gray (Fig. 2-C) rather than the characteristic brown, a color developed by some spores after 30 hr. Single-celled spores initially placed at 50 and 60% relative humidity for 12 and 24 hr, respectively, did not become two-celled until they were transferred to 100% relative humidity. When both spore types were dispersed on glass cover slips, many germinated within 24 to 48 hr at 95 and 100% relative humidity. At these humidities, the hyaline spores generally differentiated into the other spore type during germination by developing the gray color and septa. At 85 and 90% relative humidity, neither differentiation nor germination occurred by either spore type.

Survival of single- and two-celled spores after exposure for 1 to 4 days to relative humidities of 22 and 85% is shown in Fig. 3. The two-celled spores retained good germinability after exposure to both humidities. However, germination of spores exposed to 22% relative humidity was less than that of spores exposed to 85%. A similar response was noted with the single-celled spores, but these spores were much more sensitive than two-celled spores to desiccation, as after 2 days' exposure to 22% relative humidity only 10% of them germinated. Survival of the two-celled spores produced the previous year on naturally infected citrus deadwood was quite good. Germination of these overwintering spores ranged from 66 to 94% depending upon time of collection.

Discussion.—Initially, pycnidia of *D. natalensis* are immersed and difficult to detect; but as the season progresses they become more erumpent, until by the end of the summer they are embedded in a definite stroma. Pycnidia in these stromata do not discharge spores the following season, except in cases where full stromatic development does not occur the first season.

Spores of *D. natalensis*, which are usually single-celled at the time of discharge, are released from pycnidia following either wetting or drying. These conditions occur frequently during the summer in Florida when rain occurs as numerous thundershowers. Though the single-celled spores do not tolerate desiccation as well as the two-celled forms, they are sufficiently tolerant to withstand humidities below 100% before developing into the two-celled spore when 100% relative humidity again exists. Few single-celled spores are observed surrounding discharging pycnidia, except following rain, suggesting that development into the two-celled stage readily occurs under natural conditions. Once the two-celled spore is formed, it readily survives from one season to the next. Survival of the two-celled spore may be associated with the development of a secondary spore wall not present in the single-celled spore (6).

Infection of immature citrus fruit by single- and two-celled spores occurs during rainfall in the summer months when pycnidia are discharging spores. During the winter or spring, additional infection or reinfection can be caused only by two-celled spores that remain on the bark surface surrounding the pycnidia. In the spring, these spores can cause infection of immature fruit

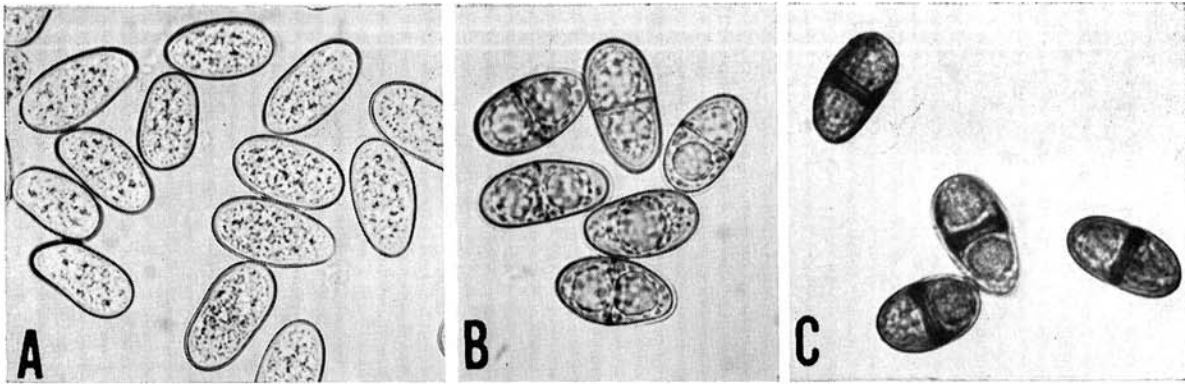


Fig. 2. A) Hyaline, nonseptate, and granular spores of *Diplodia natalensis* at discharge from the pycnidium. B) Septa and oil vacuoles exhibited in hyaline spores after 5 to 6 hr at 100% relative humidity. C) Dark-gray septate spores after 24 hr at 100% relative humidity ($\times 500$ and $\times 3$).

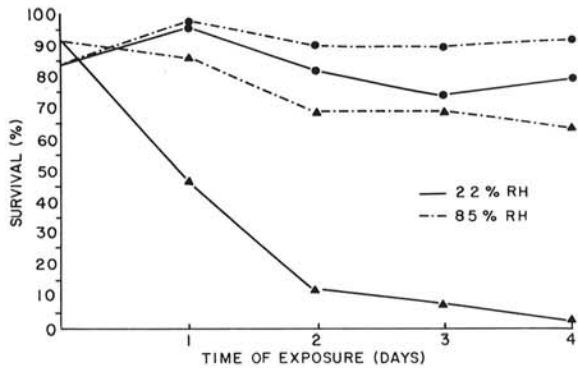


Fig. 3. Survival of single-celled ▲ and 2-celled ● spores of *Diplodia natalensis* after exposure for 1, 2, 3, and 4 days to 22 and 85% relative humidity.

formed at bloom in March or April before pycnidia become active the following June or July. The knowledge that hyaline single-celled spores of *D. natalensis* rapidly convert to dark colored two-celled spores which survive from one season to the next, in part explains why this organism so commonly exists latent on buttons of Florida citrus fruit.

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